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Evaluation of different buffered peptone water (BPW) based enrichment broths for detection of Gram-negative foodborne pathogens from various food matrices

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Abstract (max 400 words)

This study evaluated the effects of changing the composition of the pre-enrichment medium buffered peptone water (BPW) on the growth of stressed and unstressed Gram-negative foodborne pathogens in a one-broth enrichment strategy. BPW supplemented with an available iron source and sodium pyruvate, along with low levels of 8- hydroxyquinoline and sodium deoxycholate (BPW-S) improved the recovery of desiccated *Cronobacter* spp. from powdered infant formula. Growth of *Salmonella* and STEC was comparable in all BPW variants tested for different food matrices. In products with high levels of Gram-negative background flora (e.g. sprouts), the target organisms could not be reliably detected by PCR in any of the BPW variants tested unless the initial level exceeded 10^3 cfu/10g of sprouts.

Based on these results we suggest BPW-S for a one-broth enrichment strategy of stressed Gram-negative foodborne pathogens from dry products. However, a one-broth enrichment strategy based on BPW variants tested in this evaluation is not recommended for produce with a high level of Gram-negative background flora due to very high detection limits.

Keywords: pre-enrichment, one-broth strategy, supplements, resuscitation, *Salmonella*, STEC, *Cronobacter*, *Enterobacteriaceae*

1. Introduction

Key to the reliability of all methods for the detection of Gram-negative foodborne pathogens (e.g *Salmonella*, *Cronobacter* and Shigatoxin-producing *E. coli*) is the capability to recover low numbers of stressed cells from any kind of food matrix to a detectable level. These methods usually consist of three consecutive steps. The pre-enrichment step, that is common to most current detection methodologies (cultural, molecular and immunological), aims at the resuscitation/ recovery of cells in a non-selective medium such as buffered peptone water (BPW). The second step, a selective enrichment, should promote growth of the recovered target bacteria. The last step, the actual detection of the pathogen can be accomplished with either cultural or molecular methods.

Gram-negative foodborne pathogens are usually present in relatively low numbers, sometimes accompanied by a high number of closely related competitor organisms (Baylis et al., 2000). In addition, cells may be sub lethally injured by food processing or intrinsic factors of the food matrix (Edel and Kampelmacher, 1973). The purpose of the pre-enrichment step is to allow stressed target microorganisms to resuscitate and grow in either a non-selective or moderately selective environment. At this stage, the target organisms can be overgrown by the background flora in the food product due to absent selectivity of the medium, which can lead to false negative results.

The selective enrichment should promote growth of the recovered target bacteria. The necessity of applying a selective enrichment will mostly depend on the characteristics of the food product, particularly on its microbial flora. In products with a high number of competing organisms, in which the target bacteria are likely to be stressed, both a non-selective and selective enrichment step will be necessary. For products with low levels of background flora, a one-broth enrichment strategy in an unselective enrichment broth can be sufficient. Since the pre-enrichment and selective enrichment step are the main time limiting factors in

regards to turn around time for rapid methods (Baylis et al., 2000), a moderately selective one-broth enrichment strategy can decrease the total time until results are available.

The success of the pre- enrichment step is however not only depending on the food product but also on the quality of the medium. Preliminary work has shown that there are some inconsistencies in the performance of BPW and that the ISO standard formulation results in performance variations not just from manufacturer to manufacturer but also from batch to batch. A comparison of 18 different commercially brands of buffered peptone water showed differences in performance regarding buffering capacity, growth of unstressed bacteria and recovery of dry-stressed cells (unpublished data). The performance of the BPW brands depended heavily on the matrix and the organism tested. Baylis et al. (2000) compared two commercially available preparations of buffered peptone water with regards to their ability to promote recovery and growth of sub-lethally injured *S. enterica*. There was a significantly higher recovery rate stressed of cells with one brand of buffered peptone water however, the performance of BPW in food was mostly dependent on the food type with the type and level of background flora present being the most important influential factors. Strain variability also exerted an influence on the recovery of stressed cells. Different studies have investigated an improvement of enrichment media by supplementation and by modification of the conditions during enrichment such as temperature, pH, time etc. (Andrews et al., 1986). Gray et al. (2006) investigated the effect of variation between classes of casein, gelatine and peptones from different sources. They concluded that the variation in peptone has a substantial influence when looking at growth and enumeration of bacteria. For STEC, pre-enrichment methods were developed based on meat industry requirements that are, however, inadequate for the recovery of STEC from vegetables, flour and other low a_w matrices (Sata et al., 2003). Weber et al. (2009) showed that the supplementation of growth factors such as additional iron and sodium pyruvate, along with low levels of inhibiting agents primarily

against Gram-positive background flora (8- hydroxyquinoline and sodium deoxycholate), enhances the recovery rate of stressed cells.

Products containing starter cultures and probiotic cultures present a special challenge to pathogen detection. Strong acidification of the enrichment caused by lactic acid producing bacteria can lead to inactivation of target bacteria and subsequently false-negative results. Previous experimental work has shown that increased concentrations of buffer phosphates in BPW improved detection of pathogens in powders containing probiotics (unpublished data). For this reason, BPW with 3x and 6x increase in buffer phosphate concentration were included in the study. So far, there are only few studies evaluating the use of BPW with supplements in a one-broth enrichment. In particular, data obtained from experiments in the food matrix are scarce.

The aim of the current study was to compare different modifications of BPW with regard to their ability to promote growth of unstressed and stressed Gram-negative foodborne pathogens in different food matrices.

2. Materials and methods

2.1. Bacterial strains

Different *Salmonella enterica*, *Cronobacter* spp. and Shigatoxin-producing *Escherichia coli* (STEC) strains were chosen as representatives for Gram-negative food borne pathogens (Table 1). Working cultures were made from frozen (-80°C) BHI (Oxoid CM1135, Basingstoke, United Kingdom) with 20% glycerol stocks and maintained on blood agar plates (Difco Columbia blood agar base, 5% sheep blood, CM0031 Oxoid) at 4± 1°C. All strains were natural isolates, obtained from our in-house collection.

2.2 Supplementation of BPW

125 Buffered peptone water (BPW, Oxoid CM1049) was prepared according to the
126 manufacturer's instructions. BPW-S was prepared by using BPW with the following
127 supplements added before autoclaving: 40 µM 8- hydroxyquinoline, 0.5 g/l ammonium-
128 iron(III) citrate, 0.1 g/l sodium deoxycholate, 0.1g /l sodium pyruvate (all from Sigma-
129 Aldrich, Buchs, Switzerland).
130 6xBPW (quantities for 3xBPW in brackets) was prepared by addition of 7.5 g/l (3 g/l)
131 KH_2PO_4 and 17.5 g/l (7 g/l) Na_2HPO_4 (anhydrous) (Sigma-Aldrich) to BPW. 6xBPW-S
132 contains both additional buffer salts and supplements of BPW-S. Throughout this work,
133 buffered peptone water from a single batch was used to avoid lot-to-lot variations.
134
135 2.3 Recovery of dry stressed cells
136 Each ten strains of *Cronobacter* spp., STEC and *S. enterica* were grown overnight in 9 ml
137 BHI (Oxoid) at 37°C. Grown cultures were centrifuged at 13000 g for 10 min. The
138 supernatant was discarded and the pellet was re-suspended in 1 ml BPW. The cell suspension
139 with cell counts of approximately 10^9-10^{10} cfu/ml was serially tenfold diluted in BPW. 10 µl
140 of each dilution were pipetted in eight wells of a 96- well microtiter plate using one plate per
141 strain resulting in 10^7- 10^8 cfu/well in the first row. Cell counts of the culture were determined
142 with plate counts on tryptic soy agar (BD Diagnostic Systems, Heidelberg, Germany). After
143 two days of storage in a desiccator containing silica gel at room temperature, one complete
144 plate was rehydrated with 200 µl BPW, BPW-S or 3xBPW per well and incubated 16± 2 h at
145 37°C. The most probable number (MPN) of microorganisms that had survived the drying
146 process and were able to grow in the enrichment broth was determined based on the number
147 of wells in which growth was observed using the Bacteriological Analytical Manual *Online*
148 MPN table.
149 (http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm109656.htm)
150

151 2.4 Application of desiccation stress

152 In food matrices with a low a_w, pathogens are expected to have encountered desiccation
153 stress. To achieve a more realistic scenario, cells used for spiking were desiccation stressed
154 before inoculation.

155 The STEC K124 and 33, *Cronobacter* spp. E615 and E776, *S. enterica* N10 1905 and N472
156 962 strains were grown separately overnight in 30 ml BHI (Oxoid) at 37°C. After
157 centrifugation for 12 min at 4000 rpm and the supernatant was discarded. The inside of the
158 tube was dried with a sterile cotton swab. Small amounts of sterile $CaCO_3$ (Sigma-Aldrich)
159 were added to the pellet and mixed vigorously with a spatula until homogenously distributed.
160 More $CaCO_3$ was added to a final amount of approximately 15 g of powder. The powder was
161 stored in a desiccator with silica gel for two days. The homogeneity of the powder was
162 verified one day before the actual experiment by weighing five times 0.1 g of powder,
163 diluting in 0.9% saline and plating on selective agar (Rapid` *E. coli* 2 agar for *E. coli* (Biorad,
164 Marnes la Coquette, France), X.L.D agar for *S. enterica* (Oxoid), Brilliance *Enterobacter*
165 *sakazakii* agar for *Cronobacter* spp. (Oxoid)). However, plating on selective agar might have
166 resulted in lower cell counts than those actually found in the powder.

167 To obtain the desired concentration, 0.1 g of the powder with bacteria was suspended in 0.9%
168 saline, diluted if necessary and added to the enrichment. The spiking levels were checked by
169 enumeration on selective agar.

170

171 2.5 Growth of pathogens inoculated into food enrichments

172 10 g samples of skim milk powder, oat flakes, minced beef, soy sprouts and powdered infant
173 formula (PIF) were diluted 1/10 with the different enrichment media and mixed in a
174 stomacher for 30 s at medium intensity. Food products for one experiment were obtained
175 from the same supplier, if possible originating from the same batch. The samples were
176 inoculated with low levels of the target strain (aiming at 10^1 cfu in 100 ml) and incubated for

18±2 h at 37°C. For spiking with cells without application of stress (for minced beef and sprouts), an overnight culture grown in BHI was diluted to the desired concentration and added to the diluted matrix. In the case of milk powder, oat flakes and PIF, the target strains were desiccation stressed before inoculation, using the protocol described in 2.4. Skimmed milk powder was inoculated with *Salmonella*, oat flakes were inoculated with STEC and *Salmonella*, minced beef was inoculated with STEC, soy sprouts were inoculated with STEC and *S. enterica* and powdered infant formula was inoculated with *Cronobacter* spp. Inoculum levels were confirmed by plate counts on tryptic soy agar. After 4, 8, 15, 20 and 24 h of incubation, samples of each enrichment broth were diluted in 0.9 % saline and plated on the appropriate selective agar. To verify that the used food products were not naturally contaminated with *S. enterica*, *Cronobacter* spp. or STEC, negative controls were incubated and checked for the presence of pathogens by plating on selective agar or by Assurance GDS real-time PCR (Biocontrol, Bellevue, USA). Total aerobic counts and *E. coli/* coliform counts were determined in the un-inoculated samples by plating them on tryptic soy agar at regular intervals. Minced meat and sprout samples were additionally plated on Rapid` *E. coli* 2 agar (Biorad). All agar plates were aerobically incubated at 37°C for 24±2 h. The pH of the negative control was measured at regular intervals in 6xBPW and BPW-S using a pH meter (Orion Star, Thermofisher Scientific, Reinach, Switzerland). To obtain data after more then 12 h incubation an identical second pre-enrichment broth was prepared in the late afternoon, also incubated at 37°C, and sampled the next day. This approach may result in an increase of the variability.

2.6 Recovery of STEC and *Salmonella* from sprouts

Fresh soy sprouts were obtained from a local Asian supermarket. 10 g samples were diluted 1/10 with BPW, BPW-S and 6xBPW and mixed in a Stomacher for 30 s at medium intensity. Sprouts were spiked with 10, 10^2, 10^3 cfu / sample with *E. coli* (strain K124) and a naturally

nalidixic acid resistant *Salmonella* Kentucky (strain N08 2487) and incubated for 18±2 h at 37°C. Plate counts were performed to confirm the inoculation level. After incubation, the samples were diluted and plated on selective media. Samples inoculated with STEC were plated on Rapid` *E. coli* 2 agar (Biorad) and samples inoculated with *S. enterica* were plated on Luria Bertani Agar + 256 µg/ml nalidixic acid sodium salt (Sigma-Aldrich) Plates were incubated for 24 h at 37°C. In addition, presence of the pathogens was tested with the Assurance GDS real-time PCR. An un-inoculated sample was used as a negative control.

2.7 Statistical analysis

Statistical analysis was performed using IBM SPSS. The effect of the enrichment media on the resuscitation of desiccation stressed cells was compared applying one-way ANOVA.

A three-way ANOVA was used to compare the cell counts from the enriched cultures for the four enrichment media, five food matrices and the four organisms.

3. Results and Discussion

3.1. Recovery of desiccated cells

The MPN concentrations (log cfu/ml) after rehydration and incubation for the tested strains using the three enrichment media are shown in Figure 1. *Salmonella* strains were recovered to values reaching from 0.5 log MPN to 4.6 log MPN. The mean MPN counts were 3.6± 1.2, 3.7± 1.3 and 3.4± 1.3 in BPW, BPW-S and 6xBPW, respectively. Desiccated *Salmonella* strains did not show great variation in regards to the different media. Two strains remained at low cfu levels after rehydration and incubation, which indicates that growth after desiccation was more strain than media dependent. Desiccated STEC strains were recovered to highest concentrations in BPW (mean MPN count 4.1± 0.2), showing little strain variation. The mean concentrations in BPW-S and 3xBPW were up to 2.0 log MPN and 0.6 log MPN lower, respectively. There was no statistical significant difference between the media (P= 0.87).

229 Highest concentrations for *Cronobacter* strains were recorded in BPW-S (mean MPN count
230 2.5± 0.7) and 6xBPW (mean MPN count 3.3± 0.9) (P= 0.06). However, strain variability
231 resulted in differences in counts of up to 2.5 log MPN. The strains showed least resuscitation
232 in BPW (mean MPN count 3.2± 0.5).
233 The positive effect of the combined supplementation of BPW with an iron source, sodium
234 pyruvate and sodium deoxycholate on stressed *Enterobacteriaceae* was already described by
235 Weber et al. (2009). Nevertheless, recovery of desiccated STEC was significantly superior in
236 BPW without supplements (P< 0.01). One of the supplements, sodium deoxycholate, is the
237 selective component in bile salts and its use in culture media for the inhibition of Gram-
238 positive organisms and selection of Gram-negatives is well recognized (MacConkey, 1905).
239 However, the negative effect of bile on the detection of stressed pathogens has also been
240 described (D`Mello et al., (1987); Hara-Kudo et al. (2000). A concentration of 1.0 µg/ml
241 sodiumdeoxycholate in an agar medium resulted in increased lag time and growth rate of *E.*
242 *coli* and the interference with the flagellation was named as a possible reason by D`Mello et
243 al. (1987). Stephens and Joynson (1998) reported decreased recovery of acid/salt stressed *E.*
244 *coli* O157:H7 in tryptone soya broth when bile salts were added. These findings indicate that
245 bile salts with sodium deoxycholate being the main ingredient can deteriorate the recovery of
246 stressed *E. coli* cells (Stephens and Joynson, 1998).
247 Sodium pyruvate protects stressed bacteria from reactive oxygen species (MacDonald et al.,
248 1983). These can develop in the enrichment broth through auto-oxidation of reducing sugars
249 and cause decreased recovery of stressed cells (Stephens et al., 2000). Sodium pyruvate is
250 able to facilitate the repair process in injured bacteria and can replace the function of catalase
251 in these organisms. No negative effects on any of the organisms tested in our study are
252 described in literature. Therefore the observed inhibitory effect in STEC is most probably not
253 caused by this substance. 8-hydroxyquinoline (8-HQ) was added to the enrichment medium to

254 replace vancomycin as an inhibitor for Gram-positive competitors as this compound is heat
255 stable and alternatives to antibiotics are desirable. (Weber et al., 2009).
256
257
258 3.2. Maximum population density in the matrix
259 The maximum population density for each matrix with standard deviation is shown in Figure
260 2. All food matrices were inoculated with two strains of the same species and the experiments
261 were performed in triplicate. In one experiment, one food product was spiked with one strain
262 and diluted with all tested media. The spiking inoculum was identical for one experiment.
263 However, spiking levels between different replicates can differ which is the main reason for
264 the high standard deviation in some cases. When PIF was spiked with low levels of
265 desiccation stressed *Cronobacter*, highest counts were seen in BPW-S in all experiments.
266 Levels in the other enrichment media were on average 1 log cfu/ml lower than in BPW-S.
267 However, in two of the experiments, counts in 6xBPW and 6xBPW-S were as high as in
268 BPW-S. Overall, the cell counts of *Cronobacter* were not significantly different between the
269 media (P= 0.06). Weber et al. (2009) demonstrated the same improved recovery with ten
270 *Cronobacter* spp. strains. They also showed that isolation from other naturally contaminated
271 samples of different origin was enhanced
272 For skimmed milk powder, *Salmonella* strain N472 962 grew to 9 log cfu/ml in all
273 enrichment media. Variation was small in this case (mean standard deviation= 0.5). Strain
274 N10 905 grew to lower counts in all media and experiments were yielding higher standard
275 deviations (mean standard deviation= 1.3).
276 STEC strains 33 and K124 spiked into cereals grew to 9 log cfu/ml, independent of the
277 enrichment medium. In contrast to the desiccation experiments (see 3.1.), both STEC strains
278 grew to same concentration in BPW and BPW-S. When *S. enterica* was spiked into cereals,

279 growth of the target bacteria again was lower in 6xBPW and 6xBPW-S than in BPW and
280 BPW-S with high standard deviation.
281 Minced meat was spiked with low levels of unstressed bacteria. STEC strain 33 grew to levels
282 of 8 log cfu/ml to 10 log cfu/ml in BPW-S, 6xBPW and 6xBPW-S. Values in BPW were up
283 to 2 log cfu/ml lower. Minced meat was also spiked with *S. enterica*. For both strains, all four
284 media showed the same maximum population density.
285 The general outcome of the matrix experiments is that both *Salmonella* and STEC strains
286 (with the exemption of K124) did grow to comparable concentrations in all four enrichment
287 media independent of the matrix. This indicates that the food matrix balances the effects of
288 the media supplements, both positive and negative. The results also show that in the matrices
289 tested, *Salmonella*, STEC and *Cronobacter* spp. can be isolated with a one-broth strategy with
290 BPW-S. In food products in which one or more of these three targets have to be tested, one
291 enrichment could be sufficient and omission of a selective enrichment allows time saving.
292 6xBPW was included in the study because previous experimental work showed that increased
293 buffering capacity enhanced recovery of desiccated cells from products with a high number of
294 lactic acid producing bacteria (e.g. probiotic culture powders) (data not published). We did
295 not find, as expected, an additional benefit in the use of this medium for the products
296 described here.
297
298 3.3. Growth in food matrices
299 The growth of both the pathogens and the background flora was determined in BPW and
300 BPW-S. PH in the enrichment was recorded. All food matrices were inoculated with low
301 levels of the target organism.
302 The target bacteria and the background flora reached approximately the same final
303 concentration after 24 h for PIF, milk powder, oat flakes and minced meat (Figure 3). For PIF
304 and oat flakes the levels of the background flora was 1 log cfu/ml lower in BPW-S than in

305 BPW throughout the incubation period. These are the products, which have a mainly Gram-
306 positive flora. In PIF, the final number of *Cronobacter* was over 1 log higher in BPW-S. In
307 the other food matrices, there was no obvious difference between the enrichment media in the
308 growth of the selected pathogens. In PIF, oat flakes and minced meat the target organisms
309 reached their final value after 15 h. In milk powder it took 20 h until *Salmonella* reached the
310 maximum numbers.
311 Sprouts were spiked with a higher number of *Salmonella* to enable detection of the target
312 bacteria after the incubation period. It is remarkable that starting from 0 h of incubation, the
313 counts of the background flora are at least 3 log cfu/ml steps higher throughout the
314 enrichment period.
315 Background flora counts in sprouts were very high and values of 7 to 8 log cfu/g in sprouts
316 have already been published by other researches (Jinneman et al., 2012). In contrast to the
317 other matrices used in this study, the background flora of sprouts is mainly composed of
318 Gram-negative organisms and therefore the inhibiting agents in the BPW variants are not
319 effective because they were selected to supress the Gram-positive flora. Inhibiting effects of
320 the background flora on pathogens like *E. coli* O157 and *Salmonella* have been described
321 previously (Vold et al., 2000, Duffy et al., 1999). An approach explaining this behaviour is
322 the Jameson-effect. According to this model, two species compete for the resources in the
323 environment during their growth. Growth of both populations stops when the resources are
324 depleted or when metabolic products inhibiting growth have accumulated (Cornu et al.,
325 2011). It is possible that this model also applies in sprouts. However, since growth in our
326 experiments was only determined up to 24 h, the growth behaviour of *Salmonella* after this
327 time is not known.
328 In the enrichment of dry food products, pH values remained relatively stable. Only in milk
329 powder did the pH in BPW-S decrease to 5.8. BPW with increased buffer concentration did
330 not completely prevent pH decline but usually kept the pH about 1 point higher. However, pH

331 decrease did in none of the products reach a level that would inhibit growth of the target
332 bacteria.
333
334 3.4. Detection limit in sprouts
335 Since the above-mentioned experiments indicated that low levels of *Salmonella* and STEC
336 spiked into sprouts could not be recovered, the inoculation level was increased and
337 enrichments were additionally tested with real-time PCR for the presence of the pathogens.
338 These results are summarized in Table 2. *Salmonella* could not be detected by real-time PCR,
339 when sprouts were inoculated with only 10 cfu. However, *Salmonella* counts were 10^4-10^5
340 cfu/ml, which indicates that there was growth of *S. enterica* during the enrichment.
341 At an inoculation level of 10^2 cfu, PCR results for *Salmonella* were only positive in 6xBPW.
342 At an inoculation level of 10^3 cfu, *Salmonella* was detected with PCR from BPW and 6xBPW
343 and cultural isolation yielded a minimal value of $1.0x10^5$ cfu/ml. Nevertheless, levels of 4.0x
344 10^5 cfu/ml of *Salmonella* in BPW-S were not detected in the PCR, even though the test
345 protocol included an immunomagnetic separation step. Hence, at an inoculation level of 10^3
346 cfu (levels of $\geq 10^5$ cfu/ml after the enrichment step), *Salmonella* could not be consistently
347 detected. This, however, seems not to be due to the different enrichment broths which were
348 evaluated. The high number of accompanying flora and the dilution steps which are applied in
349 the PCR (only small volumes are used) are the reasons for a high detection limit, which seems
350 to be between 10^5 and 10^6 cfu/ml after the enrichment step.
351
352 Badosa et al. (2009) have evaluated ISO enrichment real-time PCR methods with different
353 selective enrichment media and found that *Salmonella* was only detected from sprouts in 50%
354 of the cases when the inoculation level was 10 cfu in 25 g. 1 cfu was never detected even
355 though a selective enrichment was performed after the enrichment in BPW. Their method was
356 able to detect 1 cfu in 25 g of other fresh produce. Filtered soy sprout extracts did result in

positive detection for all inoculation levels, which suggest that the high levels of accompanying flora caused the failure of the detection (Badosa et al., 2009). Taking the present data into account, the possible presence of a low dose of *Salmonella* in 25 g of sprouts cannot be reliably detected with a one-broth enrichment strategy. A selective enrichment is necessary to promote selective growth of the target organism and to inhibit the background flora. However, members of the common sprout flora (such as *Citrobacter* spp.) can also grow in selective broths and can be misidentified as *Salmonella* on selective agar.

STEC were neither detected with PCR nor when using Rapid` *E. coli* 2 agar with the lower inoculation levels. Isolation of the STEC strains on agar was only possible with an inoculation level of 10^3 cfu. Still, the PCR result of the enrichment in BPW was negative.

Differences in cultural detection between STEC and *Salmonella* can be explained by different selectivity of the agar media. The *Salmonella* strain was grown on LB agar supplemented with nalidixic acid which supressed growth of any background flora on the plate. On the other hand, Rapid` *E. coli* 2 agar does only select for coliforms. Since these organisms represent the dominating flora of sprouts with counts of up to 10^5 cfu/g (Becker and Holzapfel, 1997), the small number of *E. coli* present will be overgrown on the agar medium. The single enrichment of sprouts in BPW followed by real-time PCR for the top 6 serotypes (O157, O26, O145, O103, O111, O104) is the recommended ISO protocol for the detection of STEC (ISO/TS 13136:2012). However, our results strongly suggest that low levels of contamination with STEC on sprouts are missed with this procedure. Jinneman et al. (2012) found that the method as described in the U.S. Bacteriological Analytical Manual did detect low levels of *E. coli* O104 from a sprout matrix with different real-time PCR systems. Their enrichment involved addition of acriflavin, cefsulodin and vancomycin and increased incubation temperatures. However, they also reported non-isolation of the target organism on certain selective agars due to overgrowth of background flora.

4. Conclusion

Different buffered peptone water based enrichment broths were tested to evaluate their performance in a one-broth strategy for the detection of Gram-negative food borne pathogens. Desiccation stressed cells showed variable behaviour. Resuscitation of *Cronobacter* spp. was improved in BPW-S but stressed STEC cells did not show an improved recovery in pure culture experiments. When a selection of food products was spiked with low numbers of the pathogens, STEC and *Salmonella* growth was similar in all media. *Cronobacter* spp. detection from PIF was improved with BPW-S. Therefore, we suggest the use of BPW-S in a one-broth enrichment strategy for the isolation of *Cronobacter* spp. from PIF and similar products with low a_w values. Moreover, this broth can be used for the parallel enrichment of *Cronobacter* spp., STEC and *Salmonella* from different food matrices. However, the one-broth strategy was not suitable for produce with high numbers of Gram-negative accompanying microflora. Low numbers of the pathogen will be overgrown by the background flora making their detection impossible. Next should be a thorough validation of the medium according to ISO standards.

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